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IN SILICO DESIGN, ANTI-PROLIFERATIVE ACTIVITY MODELING, MOLECULAR DOCKING AND PHARMACOKINETIC PROPERTIES PREDICTION OF SOME NON-SMALL CELL LUNG CANCER (NSCLC) THERAPEUTIC AGENTS

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ABSTRACT
Lung cancer remains the leading and deadly type of cancers worldwide. It was estimated to account for about 25% of the 7 million people that died as a result of cancer-related illness every year in the world. In silico/structure-based approach was used to design nine (9) new non-small cell lung cancer (NSCLC) drugs using molecule 22 (as template for the design) identified with the best binding affinity previously reported in our work. The anti-proliferative activity of these newly designed NSCLC drugs was predicted using the best model reported in our previous work and found have better anti-proliferative activities than that of the hit compound with anti-proliferative activity of 6.069 except for compounds SCD 3, SCD 6 and SCD 7 respectively. Molecular docking was studied to investigate and explore the mode of binding of these newly designed NSCLC drugs with the active site of epidermal growth factor receptor (EGFR) kinase enzyme and found have better affinities (between 10.5 kcal/mole to 11.0 kcal/mole) than the template (molecule 22) with a binding affinity of 10.4 kcal/mole and the control gefitinib with a binding affinity of -8.0 kcal/mole, respectively. None of them was found to have more than one violation of the filtering criterion used in this study which confirms their oral bioavailability with good ADMET properties. The modeled anti-proliferative activities and binding affinities of these newly designed NSCLC drugs were found to be better than that of the template (Molecule 22) and the control (Gefitinib) used in this study. They were also found to be non-toxic with good pharmacokinetic properties.
Keywords: In silico, design, Activity, Docking, Pharmacokinetic, NSCLC.

INTRODUCTION

Lung cancer remains the leading and deadly type of all cancers worldwide (Gschwind *et al.*, 2004). Lung cancer was estimated to account for about 25% of the 7 million people that died as a result of cancer-related illness every year in the world (Chico *et al.*, 2009). Lung cancer was classified traditionally into small cell lung cancer (SCLC) and non-small cell lung cancer (NSCLC) (Turker *et al.*, 2018). NSCLC is the lethal/deadly class of lung cancer with nearly 1.5 million reported cases and less than 20% survival rate (Hizal *et al.*, 2018).

Receptor kinases (RKs) belong to the ErbB family, located on cell membranes of living organisms and played a significant role in the management of the neoplasms'/malignant tumours' physiological cycle (Zhao *et al.*, 2019). EGFR is a member of RKs which was recognised to be the most significant target for the management of neoplasm/malignant tumours, which plays a vital role in the control of cancer cell growth, proliferation and differentiation (Chan and Hughes, 2015; Zhang *et al.*, 2015).

Majority of NSCLC drugs (EGFR inhibitors) share a common pharmacophore and structure of quinazoline and acrylamide. Many NSCLC drugs (EGFR inhibitors) have been designed and developed starting from first, second and up to third generations. The first-generation NSCLC drugs such as gefitinib and erlotinib were developed to treat patients with EGFR mutation caused by the L858R mutation (Maemondo *et al.*, 2010). The first-generation NSCLC drugs (EGFR inhibitors) were reported to have a remarkable therapeutic effect in the early stage of clinical treatment, in the first year of treatment with these drugs more than 50% of patient developed resistance to these drugs by the T790M mutation

(Kobayashi *et al.*, 2005). As such, many second-generation NSCLC drugs such as afatinib and docatininb were designed and developed to manage the resistance caused by the T790M mutation. The stated objective was not achieved due serious side effects caused by the so called second generation NSCLC drugs such as diarrhea, skin rashes etc. Many third-generation EGFR inhibitors such as AZD9291 (Osimertinib) and CO1686 (Rociletinib) were then designed and synthesised to manage the developed resistance caused by the $EGFR^{T790M/\ L858R}$ mutations (Zhou *et al.*, 2009).

This work aimed at performing *in silico*/structure-based design of new NSCLC drugs with their anti-proliferative activity modeling, molecular docking and pharmacokinetic properties prediction.

MATERIALS AND METHODS

Compound selection

From our previous study Ibrahim *et al.* (2020b), virtual screening via molecular docking was performed which identified molecule 22 (Fig. 1) with the most promising affinity of -10.4 kcal/mol toward the EGFR receptor and pIC_{50} of 6.069 as the hit compound. (Ibrahim *et al.*, 2020b).

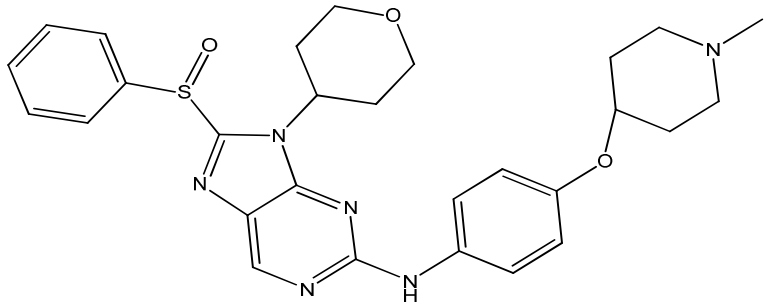


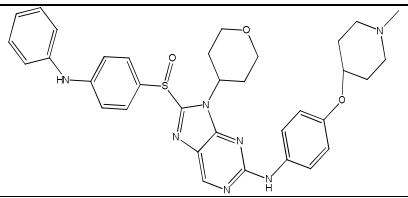
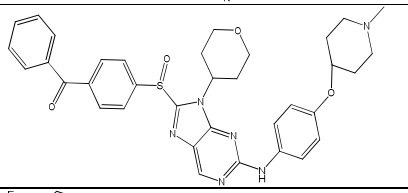
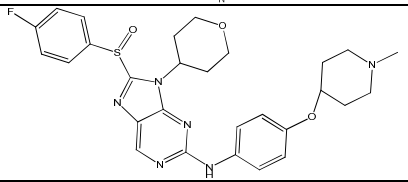
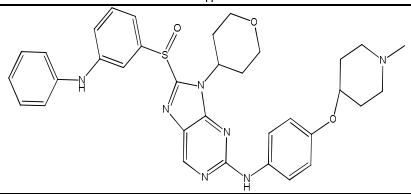
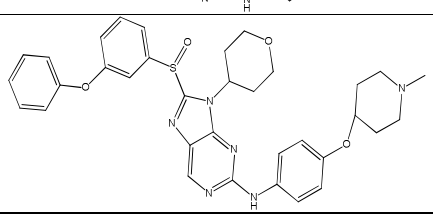
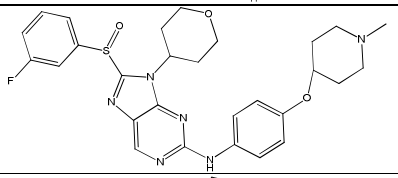
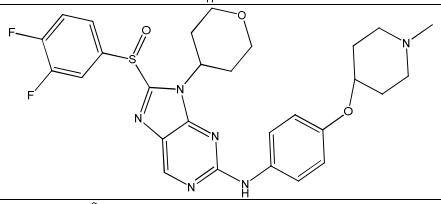
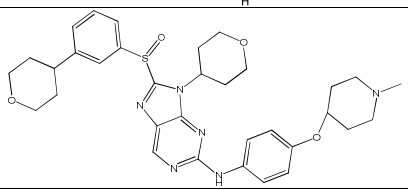
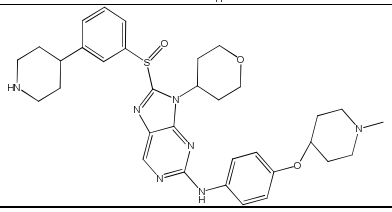
Fig. 1: The 2D structure of the identified hit compound

Design, optimum conformation search and preparations of the newly designed NSCLC drugs

Among the NSCLC drugs investigated in our previous study, molecule 22 (the best hit compound identified) was retained as template to be modified for designing new NSCLC drugs. Nine (9) new NSCLC drugs were designed by the addition of halogens, halo substituted phenyl ring, phenyl methanone and amino rings on the meta and para positions of the phenyl ring on the sulfinyl group of the template compound.

Chemdraw software was then utilized to draw the two dimensional (2D) structures of these newly designed NSCLC drugs (Table 1) (Ibrahim *et al.*, 2020c; Mills, 2006). The search for the optimum conformation of the newly designed NSCLC drugs was performed using density functional theory (DFT) at B3LYP/6-311G* level of theory. Then the stable conformations of these newly designed NSCLC drugs were then saved in pdb format (Ghamali *et al.*, 2016; Ibrahim *et al.*, 2020a).

Table 1: The two dimensional (2D) structures of the newly designed NSCLC drugs

Entry	Structures	Binding affinities (kcal/mol)
SCD1		-10.6
SCD2		-11
SCD3		-10.5
SCD4		-10.7
SCD5		-10.5
SCD6		-10.6
SCD7		-10.6
SCD8		-10.5
SCD9		-10.6

Retrieval and preparation of EGFR kinase receptor and molecular docking execution

The crystal structure of EGFR kinase domain T790M mutation in complex with AEE788 with pdb entry code **2jiu** was successfully retrieved from the RCSB protein data bank database, prepared and used in this study.

Vina of Pyrex-virtual screening tool was utilize for the docking execution of these newly designed NSCLC drugs with the active site of EGFR kinase receptor respectively (Ibrahim *et al.*, 2019). UCSF chimera software was then used to re-couple the docked newly designed NSCLC drugs and the EGFR kinase receptor and saved in pdb format for the investigation of the respective amino acids the newly designed NSCLC drugs interacted with in the active site of the EGFR kinase receptor. Discovery studio was used for the investigation of the respective amino acids the newly designed NSCLC drugs interacted with in the active site of the EGFR kinase receptor (Ibrahim *et al.*, 2020d).

Pharmacokinetic properties prediction of newly designed NSCLC drugs

SWISSADME an online web tool was utilized in predicting the drug-likeness of these newly designed NSCLC drugs following the lipinski's rule of five. While the ADMET properties of these newly designed NSCLC drugs under investigation were predicted using the pkCSM also an online web server which uses graph-based signatures to generate predictive models of central ADMET properties for drug discovery (Daina *et al.*, 2017; Hadni and Elhallaoui, 2020).

RESULTS AND DISCUSSION

Anti-proliferative activity modeling of newly designed NSCLC drugs

To further confirm the predictive performance of the model developed in our previous study (Eq. 1, which was selected and reported due to its statistical significance as compared to other models developed), the model was used to perfectly predict the pIC_{50} of these newly designed NSCLC drugs (Table 2). From the Table, It can be seen that the predicted pIC_{50} of these newly designed NSCLC drugs were better than that of the hit compound with pIC_{50} of 6.069 except for compounds SCD 3, SCD 6 and SCD 7, respectively. This further affirmed the high predicting power of the model used in modeling the pIC_{50} of these newly designed NSCLC drugs.

pIC_{50} = - 12.417755021 **(AATS7e)** + 5.879592939 **(AATS8e)** - 0.433185723 **(ATSC3e)** - 22.018131847 **(MATS7m)** + 0.333566302 **(VR3_D)** + 46.983337086. Equation 1

Table 2: The predicted pIC_{50} newly designed series newly designed NSCLC therapeutic agents.

S/No	AATS7e	AATS8e	ATSC3e	MATS7m	VR3_D	Predicted pIC_{50}
SCD1	7.611216	7.607354	-0.71719	-0.01603	30.63434	8.079382
SCD2	7.6306	7.623466	-0.52085	0.001752	31.21768	7.651513
SCD3	7.697461	7.639987	-1.25098	-0.04051	24.41977	5.897662
SCD4	7.648152	7.629845	-0.71719	-0.05005	29.96382	8.278398
SCD5	7.660484	7.669984	-1.12632	-0.03987	29.96382	8.314503
SCD6	7.712694	7.68877	-1.25098	-0.04015	24.30046	5.947483
SCD7	7.765292	7.758226	-0.06088	-0.01294	26.58752	5.350978
SCD8	7.71003	7.686867	-0.79694	-0.08749	28.8701	8.339379
SCD9	7.677397	7.659215	-0.35741	-0.08579	28.8701	8.354263

Molecular docking investigation of newly designed NSCLC drugs

The molecular docking performed was used to investigate the mode of binding interactions of these newly designed NSCLC drugs and the EGFR kinase receptor, respectively. The binding affinities of these newly designed NSCLC was found to be between -10.5 kcal/mole to -11 kcal/mole, respectively. The best three (3) among these newly designed NSCLC drugs were SCD 2 (-11 kcal/mole), SCD 4 (-10.7 kcal/mole) and SCD 6 (-10.6 kcal/mole), respectively.

SCD 2 interacted via carbon-hydrogen bonding with PRO877 (3.65096 Å) amino acid residue. The compound was not bounded only through carbon-hydrogen but also via electrostatic and hydrophobic interactions with ARG841 (2), ASP837, MET790, PHE723, PRO877, ALA743 (2), VAL726 (2), LEU844, LYS745 and CYS797 amino acid residues, respectively.

SCD 4 the second best designed compound was observed to bind through six hydrogen bonds (four conventional hydrogen bonds and two carbon-hydrogen bonds) with ARG841 (2.91595 Å), ARG841 (2.93229Å), ASP837 (2.07218 Å) LYS745 (2.57809 Å) LEU788 (3.7258 Å) and PHE723 (3.46666 Å) amino acid backbones. Beside the conventional and carbon-hydrogen bonds, it formed electrostatic and hydrophobic interactions with ARG841 (2), LEU844, PHE723 (2), ALA743, LYS745, MET790, VAL726 and LEU858 amino acid residues, respectively.

The following amino acid residues MET793 (2.68907 Å), MET793 (2.11441 Å) and THR854 (2.28229 Å) in the active site of the receptor enzyme bounded with SCD 6 via three (3) conventional carbon hydrogen respectively. It was also observed to interact with LEU718 (2), LEU844, MET766, VAL726 (2), ALA743, LYS745 and MET790 amino acid residues in the active site of the receptor enzyme via nine (9) hydrophobic interactions, respectively.

These newly designed NSCLC drugs were observed to have more affinity toward their receptor enzyme than that of the template (-10.4 kcal/mole) and the control Gefitinib (-8.0 kcal/mole). The visualized Two-dimensional structures of the best three (3) designed NSCLC drugs are presented in Fig. 2, 3 and 4 respectively.

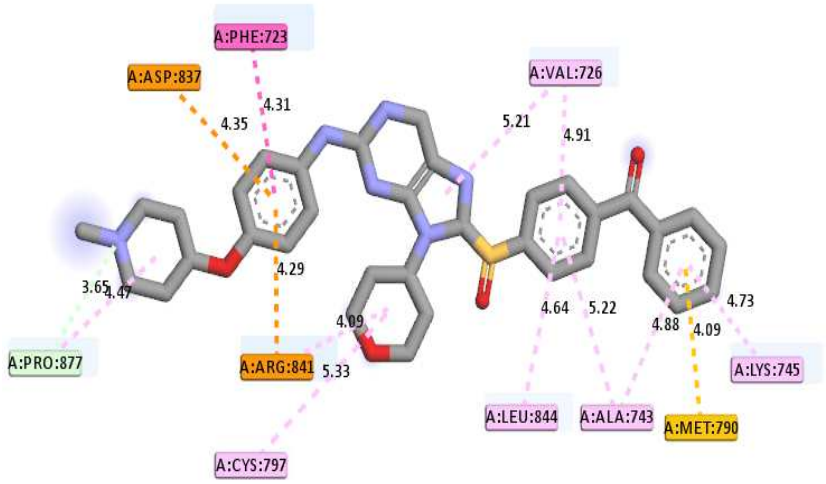


Fig. 2: Two-dimensional structure of SCD2-Receptor using discovery studio

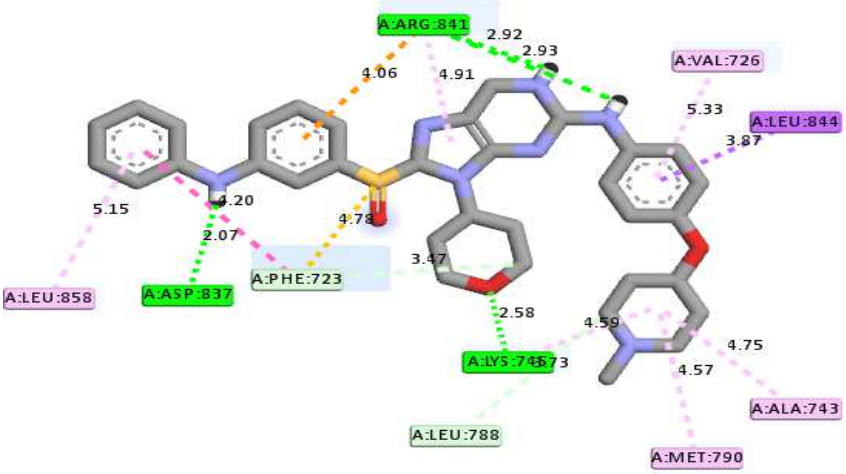


Fig. 3: Two-dimensional structure of SCD4-Receptor using discovery studio

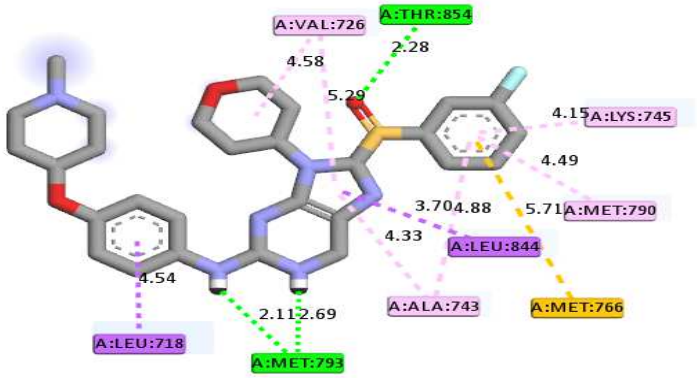


Fig. 4: Two-dimensional structure of SCD6-Receptor using discovery studio

Pharmacokinetic properties of newly designed NSCLC drugs

The Lipinski's rule of five filtering criterion was applied for the evaluation of the predicted drug-likeness properties of these newly designed NSCLC drugs (Table 3). All these newly designed NSCLC drugs were found to break only one (1) of the Lipinski's rule of five (molecular weight > 500). The molecular weight of these newly designed compounds was greater than 500 which might be as result of the addition of phenyl rings in the course of the design. The number of hydrogen bond donors and acceptors for all were less than 5 and 10, respectively. The TPSA and the WLOGP value were less than 140Å and 5, respectively. The synthetic accessibility scores of these newly designed NSCLC drugs were in the easy portion of the scale (4 to 5). It means that there is high tendency these newly designed NSCLC can be synthesize in the laboratory. On that basis, the newly designed compounds predicted to be drug-like compounds, orally bioavailable and active (Hosen *et al.*, 2017; Khan *et al.*, 2019).

Table 3: The drug-likeness of newly designed NSCLC drugs

Entry	MW	TPSA	WLOGP	No. of H-bond donors	No of H-bond acceptors	RO5 violations	Synthetic Accessibility
SCD1	623.77	125.64	4.79	2	7	1	5.01
SCD2	636.76	130.68	4.28	1	8	1	4.91
SCD3	550.65	113.61	3.61	1	8	1	4.48
SCD4	623.77	125.64	4.79	2	7	1	5.11
SCD5	624.75	122.84	4.84	1	8	1	5.09
SCD6	550.65	113.61	3.61	1	8	1	4.46
SCD7	568.64	113.61	3.16	1	9	1	4.5
SCD8	616.77	122.84	3.94	1	8	1	5.04
SCD9	615.79	125.64	4.13	2	8	1	5.07

MW: Molecular weight, TPSA: Total Polar Surface area, H-Bond: Hydrogen Bond and RO5: Rule of five

The ADMET properties predicted for these newly designed NSCLC drugs are shown in Table 4, respectively. From Table 4, the intestinal absorption values for these newly designed compounds was between 91.247% and 100%, as the values were found to have passed the threshold value of 30%, this shows that these newly designed compounds have high human intestinal absorption properties. The BBB permeability (Log BB) values of these newly designed compounds ranged between -1.503 and -1.973 respectively, which implies that all can permeate through the brain. The CNS permeability (Log PS) for all of these newly designed compounds was > -2 which are considered to penetrate the central nervous system. More so, these newly designed compounds were found to be both substrate and inhibitors of CYP3A4 which confirmed their metabolic properties and they were found to have high values of total clearance but within the accepted limit of a drug molecule in the body. Furthermore, all the newly designed NSCLC drugs were non-toxic, thereby predicting their high absorption, distribution, metabolism, excretion and low level of toxicity properties, respectively. In general, all these newly designed NSCLC drugs were predicted to have good pharmacokinetic profiles (Hosen *et al.*, 2017; Khan *et al.*, 2019).

Table 4: The ADMET properties of newly designed NSCLC drugs

S/N	Absorption	Distribution		Metabolism							Excretion	Toxicity
	Intestinal absorption	BBB permeability	CNS permeability	CYP Substrate		CYP Inhibitors					Total clearance	AMES Toxicity
		Log BB	Log PS	2D6	3A4	1A2	2C19	2C9	2D6	3A4		
1	96.569	-1.544	-3.191	No	Yes	No	Yes	Yes	No	Yes	0.41	No
2	100	-1.851	-3.344	No	Yes	No	Yes	Yes	No	Yes	0.351	No
3	92.331	-1.772	-3.334	No	Yes	No	Yes	Yes	No	Yes	0.342	No
4	96.69	-1.55	-3.21	No	Yes	No	Yes	Yes	No	Yes	0.332	No
5	99.968	-1.852	-3.281	No	Yes	No	Yes	Yes	No	Yes	0.184	No
6	92.331	-1.772	-3.334	No	Yes	No	Yes	Yes	No	Yes	0.286	No
7	91.247	-1.973	-3.381	No	Yes	No	Yes	Yes	No	Yes	0.322	No
8	100	-1.75	-3.227	No	Yes	No	Yes	Yes	No	Yes	0.434	No
9	91.4	-1.503	-3.26	No	Yes	No	Yes	Yes	No	Yes	0.43	No

BBB: Blood-brain barrier, CNS: Central Nervous System and CYP: Cytochrome

CONCLUSION

In conclusion, these newly designed NSCLC therapeutic agents were found to have higher anti-proliferative activities and binding affinities toward their target (EGFR kinase enzyme) than the template (Molecule 22) and the control (Gefitinib) used in this study. They were seen to possess drug-like properties by non-violating more than 1 of the filtering criterion used (the Lipinski's rule of five) meaning they were predicted to be orally bioavailable. More so, they were also seen to have good ADMET properties and none of them was found to be toxic. Furthermore, based on their synthetic accessibility they can be synthesize in the laboratory. Therefore, this study suggests that these newly designed NSCLC therapeutic agents should be synthesize most especially those with higher predicted anti-proliferative activity than the template among them.

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